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THE UNIVERSITY OF CHICAGO
FOUNDED BY JOHN D. ROCKEFELLER

A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
OF THE UNIVERSITY OF CHICAGO FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

(DEPARTMENT OF PHYSIOLOGY)

BY
RALPH W. WEBSTER

REPRINTED FROM THE DECENNIAL PUBLICATIONS
FIRST SERIES VOLUME X

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A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES

RALPH W. WEBSTER

I. INTRODUCTION

THE following paper is, as the title indicates, intended to be a contribution to the physical analysis of phenomena of absorption by living tissues. That the older experiments on absorption could not lead to any satisfactory explanation of the processes involved seems evident from the fact that only recently has there been discovered one of the most fundamental theories concerning the exchange of liquids separated by membranes, more or less semi-permeable. Only such papers can be expected to throw a light on this subject as take cognizance of this theory of osmotic pressure.

Van't Hoff, applying certain facts brought out by Traube and Pfeffer regarding the influence of semi-permeable membranes upon processes of osmosis, showed that substances in solution obey the ordinary laws of gases, as brought forth by Boyle, Henry, Gay-Lussac, and Avogadro. In consequence of this similarity between gases and substances in solution, the latter will exert a pressure upon the walls of a containing vessel equal to the pressure which the dissolved substance would exert were it present in the gaseous form under the same conditions of temperature and molecular aggregation. Whether this pressure, which van't Hoff calls" osmotic pressure," be due to the impacts of the dissolved particles against the walls of the containing vessel, as the kinetic theory of gases would demand, or whether it be an expression of the attraction of the dissolved particles for water, concerns us, in these experiments, only in so far as our work has to do with the dynamics of the process of absorption.

From these facts it is evident, as van't Hoff shows, that the pressure of a substance in solution depends both upon the concentration of the substance and upon the temperature at which the observation is made. By the concentration we mean, not the number of molecules, as such, contained in a definite amount of the solvent, but, rather the total number of "active" particles contained in a definite (usually 1 liter) amount of solvent. This fact was made clear by the endeavor to collate the pressures of various salt solutions with those of organic substances, or, in other words, of electrolytes with those of non-electrolytes. Clausius had shown that the molecules of substances conducting electricity, viz., of electrolytes, are dissociated into ions, which have a movement independent of one another. Arrhenius, in his work on *Dissociation of Substances Dissolved in Water*, advanced the hypothesis that the molecules of substances in solution suffer a dissociation into their electrically-charged ions (an ion being considered as an atom, or group of atoms, carrying an electric charge + or −, according

to the electric nature of the element). This conception of Arrhenius gave the new idea that the dissociation was due to the mere dissolving of the electrolyte in the water, and not, as Clausius had claimed, to the action of the electric current. This electrolytic dissociation of substances in watery solution is by no means complete at every concentration, but increases with the dilution until, at infinity dilution, a complete dissociation of the molecules into their respective ions takes place. We have, therefore, in a watery solution of an electrolyte two kinds of molecules — the active (electrically dissociated), and the inactive (non-dissociated). Inasmuch as the ions of the dissociated molecules each exert pressure, we may readily understand how the osmotic pressure of a substance in watery solution will show a much higher value as the degree of dissociation becomes greater.

The bearing of the theory of osmotic pressure upon phenomena of absorption is easily seen to be of the first magnitude. If a substance in watery solution be separated from the pure solvent by a membrane permeable to the solvent, but not to the solute, we have the conditions necessary for the solving of problems of osmosis. In such a case, as van't Hoff showed, water will pass into the solution, and, after a time, will establish a condition of equilibrium due to the pressure of the water which enters in minimal quantities. Of course the water, under such circumstances, does not give rise to the osmotic pressure measurable by a manometer, inasmuch as it is present on both sides of a membrane permeable to it. The pressure is, in this case, due solely to the dissolved particles, and may be explained by the kinetic theory, or the water-attraction of the parts dissolved.

If, instead of a membrane strictly impermeable to the dissolved particles, we have one which allows the passage of some, at least, of the dissolved particles we have a slightly different condition of affairs. In such a case the osmotic pressure will be a minimum and the process will resolve itself into one of diffusion, as the membrane being permeable to the solvent and solute, will not in any way hinder the diffusion process. However, if we add to such a condition the further complexity of two solutions separated by a membrane, freely permeable to the solvent and difficultly so for the solute, we have the conditions as they exist in the various cells of the body.

Experiments on absorption of liquids, or, in other words, upon processes involved when the conditions stated above exist, have been made on organized as well as unorganized material. The tissues chiefly involved in such work have been red-blood corpuscles, muscles, and intestines; while the unorganized material has been that known under the general name of colloid matter, including, here, gelatine, albumin, sodium-oleate, silicon-dioxide, etc. In the present work I have confined myself chiefly to the effects of solutions of various electrolytes and non-electrolytes upon absorption by muscular tissue. However, a few introductory experiments upon red blood-corpuscles were made, as it had been shown by various workers that absorption by these cells obeys the laws of osmotic pressure to a large extent, while my results on muscular tissue seem to show that variations in osmotic pressure cannot account, entirely, for phenomena noted in the latter case.

II. EXPERIMENTS ON RED BLOOD-CORPUSCLES

IT seems to be generally agreed that these cells may be regarded as systems surrounded by semi-permeable membranes. If two solutions of different osmotic pressure be separated by a more or less elastic, semi-permeable membrane, an attempt is made to equalize the pressure on each side of this membrane, with the result that phenomena occur of swelling or of shrinking of the cell in question.

Donders, Hamburger, Koeppe, Gryns, Hedin, Overton, Roth, and others have concluded, from their work on these corpuscles, that the phenomena mentioned above depend on the difference of osmotic pressure between the solution outside and that inside the corpuscular membrane, which membrane may be regarded as a thickening of the protoplasm of the corpuscle. So convinced is Koeppe of the rôle of osmotic pressure in life phenomena that he gives expression to the generality: "We cannot imagine a single phenomenon in the living organism in which osmotic pressure may not have a share."

One of the most important points to be considered in this discussion of effects of difference in osmotic pressure is that of the permeability of the corpuscle to the molecules, or ions, of the substances investigated. If it can be shown that the corpuscle be permeable to these particles, then, of course, the laws of diffusion replace those of osmotic pressure. The process, in this latter case, would then resolve itself simply into a discussion of the relative rate of diffusion of the various particles in question.

Certain facts and experiments show, rather conclusively, that the corpuscular membrane is to be regarded as a semi-permeable membrane, permeable to the molecules of water (and to ions, or molecules, of certain inorganic and organic substances) but impermeable, to a large extent, to substances in solution both as regards the solution inside and that outside the membrane. As Koeppe states, the red corpuscles contain no sodium chloride, while the serum contains 5.546 gms. in 1,000 gms. On the other hand, the corpuscles contain 3.679 gms. of potassium chloride, while the serum contains only .39 gms. in 1,000 gms. These facts, in themselves, show that the corpuscle is impermeable to the ions of the salts in question, else an equilibrium would be established on both sides of a permeable membrane. Gryns, Eykman, Hedin, and Oker-Blom have carried out extensive series of experiments to show the relative permeability of the corpuscle to various substances. Gryns, in his work on *Influence of Dissolved Substances upon Red Blood-Corpuscles in Connection with Phenomena of Osmosis and Diffusion*, gives a tabular list of substances to which the corpuscle is permeable and impermeable. Among these we find salts of metals, certain NH_4 salts, such as sulphate, nitrate, phosphate, and tartrate, glycocoll, sugar, etc., to be incapable of penetrating the corpuscle; while other NH_4 salts, such as chloride, bromide, and oxalate, alcohol, glycerine, urea, etc., easily penetrate this cell. Hedin and Oker-Blom, working with methods different from those of Gryns, confirm his results.

A fact of the utmost importance in this discussion of the permeability of the corpuscular membrane, as well as of any other membrane, to different substances is the following: A removal of free ions from a solution is possible only when ions of oppo-

site electric charges are removed together, because the electric charges of the ions hinder the free movement from the solution. Therefore, if a semi-permeable membrane is impermeable to one ion of a molecule it is impermeable to the other, for, if this were not true, a separation of positive or negative electricity would take place.

In solutions, therefore, of any of these substances to which the corpuscular membrane is impermeable, phenomena of swelling or of shrinking occur if the osmotic pressure of the outside solution be less, or more, than that of the solution within this membrane. This osmotic value of the solution within the corpuscular membrane varies, according to Hamburger, between that of a .75 per cent. NaCl solution and that of a .9 per cent. NaCl solution.

If, now, we place the corpuscles in solutions of substances to which they are permeable, osmotic pressure effects play no rôle whatever, even though the concentration of the outside solution be of higher, equal, or lower value than that inside the membrane. We find that, in these latter cases, phenomena of swelling or shrinking do not occur. As Hedin points out, the blood corpuscles do not, under such circumstances, obey the laws of osmotic pressure, and, as a result, they give up their haemoglobin to the outside solution.

Hamburger, in his earlier experiments, had attempted to apply a method based upon that of De Vries's classic work on plasmolysis in plants. He used, in these experiments, the point at which the corpuscle began to lose its haemoglobin as his isosmotic point. He maintained that the corpuscles were, to a high degree, permeable to the salts in question. As a result of this, he says, an equilibrium is established between the osmotic pressure on each side of the membrane. These ideas are, as shown above, totally at variance with the theory of osmotic pressure as advanced by van't Hoff.

In his work Oker-Blom advanced the idea that entirely erroneous conceptions of the osmotic equivalents of the corpuscles must creep in by use of solutions of salts in water. He advocates the use of solutions in serum, inasmuch as the variation introduced in osmotic pressure is, then, referable to the salt added, without considering the consequent lowering of the concentration of the blood on addition of a watery solution of the salt. He shows that, in previous work along these lines, there has always been a question of reciprocal action between the substances present in the animal cells and those with which the cells are brought into contact. In view of such conditions, he says, only the total osmotic pressure of the fluids under observation has been considered, while, from his work, it seems quite evident that the entrance of a substance into a red blood-corpuscle is, clearly, under the influence of the partial osmotic pressure of the substance added, inasmuch as no great increase in total osmotic pressure has been caused by this addition.

My own experiments, few in number, were planned simply with the idea of showing, if possible, that we are dealing, in the case of the red corpuscle, with osmotic pressure effects to a large extent. These experiments have no claim to originality, but are confirmatory of the work previously mentioned. The method followed in this work is a slight modification of Hedin's and Koeppe's hæmatokrit method. Mixtures

were made of defibrinated blood and of isosmotic salt solutions in various dilutions. In some cases equal parts of each (blood and salt solution isosmotic with blood) were taken. In other cases, 2, 3, 5, and 10 parts of solution to 1 of blood were used. These mixtures were placed in dishes containing, approximately, 25 c.c., and allowed to stand for intervals of 1, 3, 6, and 24 hours. At the end of each interval readings were taken by the hæmatokrit method, using one revolution per second for 3 minutes. By this method the percentage relation of corpuscles to serum was obtained. These experiments can have no further value than a comparative one, inasmuch as the osmotic pressure of the mixtures was not determined, and thus the concentration of the solution acting on the corpuscle was unknown. However, as solutions of salts isosmotic with the blood serum and, therefore, with one another were used, we may readily show whether osmotic pressure differences account for phenomena noted or whether we are dealing with specific ionic effects. Below will be found a table embodying the results of these experiments:

TABLE I

Substance	Concentration	Blood Dilution		Per Cent. Vol. of Corpuscles in 3 Hours	Substance	Concentration	Blood Dilution		Per Cent. Vol. of Corpuscles in 3 Hours
		Solution	Blood				Solution	Blood	
NaCl	$\frac{5}{4}$ m	10	5	10	KCl....	$\frac{1}{4}$ m	10	5	11
NaCl	$\frac{5}{4}$ m	10	10	19	KCl....	$\frac{1}{4}$ m	10	10	22
NaCl	m	10	5	8	KCl....	$\frac{1}{8}$ m	5	5	17
NaCl	m	10	10	15	KCl....	$\frac{1}{8}$ m	10	5	10
NaCl	$\frac{1}{2}$ m	10	5	9	KCl....	$\frac{1}{8}$ m	15	5	7
NaCl	$\frac{1}{2}$ m	10	10	20	KCl....	$\frac{1}{8}$ m	10	10	25
NaCl	$\frac{1}{4}$ m	10	5	12	KCl....	$\frac{1}{8}$ m	60	20	21
NaCl	$\frac{1}{4}$ m	10	10	22	KCl....	$\frac{1}{16}$ m	10	5	dissolution
NaCl	$\frac{1}{8}$ m	5	5	17	KCl....	$\frac{1}{16}$ m	10	10	32
NaCl	$\frac{1}{8}$ m	10	5	11	KCl....	$\frac{1}{32}$ m	10	5	dissolution
NaCl	$\frac{1}{8}$ m	15	5	9	KCl....	$\frac{1}{32}$ m	10	10	7
NaCl	$\frac{1}{8}$ m	10	10	26	$CaCl_2$..	$\frac{1}{8}$ m	20	20	33
NaCl	$\frac{1}{8}$ m	20	20	42	$CaCl_2$..	$\frac{1}{8}$ m	60	20	12
NaCl	$\frac{1}{8}$ m	60	10	10	$CaCl_2$..	$\frac{1}{8}$ m	60	10	8
NaCl	$\frac{1}{8}$ m	60	20	19	$CaCl_2$..	$\frac{1}{8}$ m	5	5	15
NaCl	$\frac{1}{16}$ m	10	5	dissolution	$CaCl_2$..	$\frac{1}{8}$ m	10	5	12
NaCl	$\frac{1}{16}$ m	10	10	30	$CaCl_2$..	$\frac{1}{8}$ m	15	5	8
NaCl	$\frac{1}{32}$ m	10	5	dissolution	NH_4Cl.	$\frac{1}{8}$ m	5	5	dissolution
NaCl	$\frac{1}{32}$ m	10	10	8	NH_4Cl.	$\frac{1}{8}$ m	10	5	dissolution
KCl	$\frac{5}{4}$ m	10	5	6	NH_4Cl.	$\frac{1}{8}$ m	15	5	dissolution
KCl	$\frac{5}{4}$ m	10	10	16	NH_4Cl.	$\frac{1}{8}$ m	10	10	dissolution
KCl	m	10	5	5	Urea ...	.2286 m	5	5	dissolution
KCl	m	10	10	12	Urea ...	.2286 m	10	5	dissolution
KCl	$\frac{1}{2}$ m	10	5	9	Urea ...	.2286 m	15	5	dissolution
KCl	$\frac{1}{2}$ m	10	10	18	Urea ...	.2286 m	10	10	dissolution

A study of this table shows two facts quite clearly. The first is, that we have two classes of substances represented here. The one class, to which the corpuscular membrane is permeable, embraces NH_4Cl and urea; the other class, to which the

membrane is impermeable, includes NaCl, KCl, and $CaCl_2$. The second fact brought out by this table is, that the phenomena of swelling and shrinkage of the corpuscles, when placed in salt solutions of various dilutions, are under the influence of osmotic pressure differences and not under the influence of specific ionic effects.

These experiments confirm in a simple way the results of Gryns and Oker-Blom, by showing the permeability of the corpuscle to various substances and the impermeability to others.

III. EXPERIMENTS ON MUSCULAR TISSUE

The work on the influence of osmotic pressure upon phenomena of swelling in muscle has been largely carried out by Professor Loeb and his pupils. He found, in his work on the effects of ions, that the addition of a small amount of a dilute acid or alkali to a physiological salt solution caused a muscle, immersed in such a solution, to take up water, or, in other words, to gain in weight to a considerable extent. Further investigation showed that this effect was due to the number or concentration of free H ions in the former and of free OH ions in the latter case. Miss Cooke, working under the direction of Professor Loeb, found that a muscle immersed in $\frac{1}{8}$ m NaCl for some time (eighteen hours) neither gained nor lost in weight. She therefore assumed that such a solution was isosmotic with the muscle-plasma. This conclusion is open to some of the objections raised against the work on red corpuscles, with the addition that here we are dealing with a membrane, the sarcolemma, which allows the passage of ions in both directions, and hence does not as fully obey the laws of osmotic pressure as do the corpuscles. As will be shown later, the sarcolemma is permeable to Na and K ions, and hence we should expect the same result with isosmotic solutions of Na and K salts. This, however, is not the case, as Loeb himself points out in his further work along this line. He shows that a muscle immersed in $\frac{1}{8}$m NaCl for eighteen hours gains only slightly (5 per cent.) in weight; one immersed in $\frac{1}{8}$m KCl gains 50 per cent., while in $\frac{1}{10}$m $CaCl_2$ it loses 20 per cent. He advances as explanation the hypothesis that we have to do with a chemical combination of the ions of the salt with the proteid molecule, giving us what he terms "ion-proteids," which he assumes show similar reactions, especially as regards their fluid absorbing power to those of soaps.

From the study of the work of Hofmeister, Lewith, van Bemmelen, Pauli, and Hardy on effects of salts upon colloids, we obtain a much clearer insight into the properties, both physical and chemical, of the body-colloids. Hofmeister attempts to show that the albumin and globulin precipitating power of salts is due to their dehydrating power, giving as his reasons for this view (1), the precipitating power remains practically the same for action of different salts on various colloids (the degree of dissociation of the salts being a great factor); (2), this power goes parallel to other physical and chemical properties of salts which are dependent on dehydrating power. In his work on the phenomena of swelling he calls attention to the fact that we must take cognizance of three forces, viz.: capillary imbibition, endosmotic imbibition, and

molecular imbibition. Through these forces, he is led to believe, the absorption of water and of salts is controlled, it being quite evident that the absorption of water and salts goes on quite independently the one of the other.

Van Bemmelen, in his work on absorption by colloids, has shown that inorganic colloids, such as SiO_2, $Fe(OH)_3$, etc., have quite a similarity to organic colloids. The absorption of water and of salts from solutions is dependent on the nature of the substance in solution as well as on the concentration of this substance. Absorption by inorganic colloids, he states, depends on several factors, (1) structure of colloid, (2) modification brought about in this structure through gel-formation, heat, etc., (3) vapor-tension (osmotic pressure) of fluid to be absorbed, (4) temperature, (5) kind of solvent. Great interest attaches itself to his work on the conversion of a hydrosol into a hydrogel. He declares that salts (with strong acids) of trivalent metals have the strongest coagulating power, then follow in turn the salts of bivalent and univalent elements. This fact has been elaborated by Hardy into the law that "the coagulating power of salts of elements increases as the 2nd, 3rd . . . . power of the valence of that element." In this connection Hardy states that, in general, electrolytes have an effect while non-electrolytes have none; a statement quite in harmony with those made by Loeb, Lingle, Moore, Mathews, and others concerning the action of electrolytes and non-electrolytes on organic colloids.

In order to test the conclusions of the various workers and to show, further, the action of various ions as well as the rôle of osmotic pressure in the phenomena of absorption of liquids by muscular tissue, this work was undertaken at the suggestion of Professor Loeb.

IV. MATERIAL AND METHOD

Owing to the fact that it is readily accessible, that any variations in its condition may be easily controlled, and that experiments with it may be accurately carried out, the gastrocnemius muscle of the frog was selected as the research material for these experiments. The muscle is removed from the leg, care being taken not to injure the muscle substance, not to use for experimentation muscles which have been very active previous to removal, and to exclude those muscles which show bruises or hemorrhagic areas. After being carefully dried with sheets of filter-paper, the muscle is placed between watch-glasses and accurately weighed. Thus weighed the muscle is placed in a dish containing approximately 25 c.c. of the solution of the substance whose action is under investigation. The concentration of the solutions varied within rather wide limits, inasmuch as it seemed desirable to ascertain the effects of various concentrations of the same substance, as well as the same concentration of various substances. The working basis of the concentration was made that of $\frac{1}{8}$ m NaCl, as this had been previously shown by Miss Cooke to be isosmotic with the muscle-plasma. The concentrations of the various solutions were graded, starting with $\frac{5}{4}$ m., following with m, $\frac{1}{2}$ m, $\frac{1}{4}$ m, $\frac{1}{8}$ m, $\frac{1}{16}$ m, and ending with $\frac{1}{32}$ m, This range seemed necessary in order to give ample scope to the study of hyper-, iso-, and hypo-tonic solutions. In the later experiments only solutions isosmotic with $\frac{1}{8}$ m NaCl were used, as it appeared possible,

by this means, to arrive at more definite conclusions concerning the effect of osmotic pressure. In calculating the strength of a solution which shall be isosmotic with a known solution, it is necessary only to make use of the simple formula:

$$y = \frac{P}{22.35\,(1 + (n-1)\,a)}$$ in which

P = osmotic pressure of known solution.

y = strength of unknown solution in terms of normal solution.

$m = n,\ 2n,\ 3n$, etc., according to basicity of salt.

$n_i^!$ = number of ions into which the molecule of salt is dissociated.

a = degree of dissociation of solution of same molar concentration as known solution.

After remaining in such solutions for intervals of one, three, six, and twenty-four hours, the muscle is taken out, carefully dried, as before, and weighed. In the drying of the muscle with filter paper, two errors are prone to creep into the work and should be guarded against. The first of these is a positive one and consists in not removing all of the fluid adhering to the surface. The second is a negative one and consists in exerting undue pressure on the muscle, thereby drying out the surface layer of muscle, and causing an increase of pressure upon the inner layers. This latter error is greater, of course, the smaller the muscle, inasmuch as we have a much larger surface in proportion to the total mass of muscle. The percentage gain or loss in weight of the muscle (as ascertained by comparing the weight before immersion in the solution, with the weight after removal from the same solution), gives the absorption. In the experiments detailed here the gain or loss is interpreted as meaning an increase or decrease in the amount of water in the muscle, although it is more than probable that the change is due to interchange of ions through the sarcolemma and an ultimate establishment of equilibrium between partial osmotic pressures on both sides of this membrane.

V. EFFECTS OF NON-ELECTROLYTES

We understand by non-electrolyte any substance which exists in solution in the molecular and not in the ionic form. Such substances, when in solution, do not conduct electricity and do not show irregularities in lowering of the vapor tension of the solution. They obey the laws of osmotic pressure, however, and therefore give us opportunity of studying the osmotic effects in absorption phenomena, although the ionic effects are excluded by their use. The nonelectrolytes used in these experiments were water, cane sugar, and urea. The concentrations were, except naturally in case of water, made isosmotic with the various NaCl solutions to be used later.

a. Absorption from Redistilled Water.—A gastrocnemius muscle placed in redistilled water goes through the following phases of absorption:

Absorption			
1 h.	3 h.	6 h.	24 h.
+34	+56	+72	+67

These results show, beyond a doubt, that in this case we are dealing with a purely osmotic phenomenon. The natural result of placing a solution of salts (the

muscle-plasma) upon one side of a membrane partially permeable (as will be shown later) to these salts, and, on the other side of this membrane, pure water to which the membrane is more or less freely permeable, is a rapid passage of water into the salt solution and a very slow passage of ions into the water. As the time of the experiment is extended, we find that a point is reached when the outside concentration of salt may be greater than that inside. In consequence of this condition water passes from the muscle into the solution. Possibly the osmotic pressure within the muscle may be increased by the production of sarco-lactic acid, in which case the steady increase in absorption may be accounted for. Hofmeister states that a muscle immersed in water absorbs a certain amount, which cannot exceed a limit known as the "maximum of swelling," which limit is dependent on the amount of (1) capillary imbibition, (2) osmosis, and (3) adsorption. Our results show that a large part of the absorption from water is due to direct endosmotic imbibition.

b. Absorption from Cane Sugar Solutions.—The absorption noted when a muscle is placed in solutions of various concentrations of cane sugar is as follows:

TABLE II

Concentration	Isosmotic	Absorption			
	NaCl Sol.	1 h.	3 h.	6h.	24 h.
1.6745 m	m	−40	−53	−57	− 53
.8667 m	$\frac{1}{2}$ m	−27	−35	−35	− 22
.4465 m	$\frac{1}{4}$ m	−12	−12	−12	− 2
.2286 m	$\frac{1}{8}$ m	− 1	+ 5	+ 9	+10
.1168 m	$\frac{1}{16}$ m	+11	+22	+31	+41
.05906 m	$\frac{1}{32}$ m	+26	+47	+64	+87

It is quite evident from the above table that the absorption is dependent on certain main factors, viz.: (1) time of action of the solution, (2) concentration of the solution, (3) nature of the substance in solution. It will be observed that the effects during the first hour are much more marked, relatively speaking, than during the other intervals. As will be shown later, the effects are not purely those due to the differences in osmotic pressure on both sides of the sarcolemma, but are, rather, especially in the case of salts, a mixture of osmotic and specific molecular or ionic effects, together with effects due to metabolic changes. It may, however, be said here, that in the earlier intervals osmotic pressure effects are much the more prominent.

The effect of concentration of the solution may also be noted in the above table. It appears to be a general rule that hyper-tonic solutions bring about a negative absorption during the first intervals, while the iso- and hypo-tonic solutions cause a positive absorption. This negative absorption of the first few hours may or may not change in the later intervals to a positive one, according to the nature of the substance in solution.

Along with these factors which influence the action of solutions in phenomena of

absorption, must be mentioned certain conditions peculiar to the muscle which play a minor rôle in the phenomena: (1) difference in size (surface) of the muscles used may cause some variation in the average of absorption from any solution, inasmuch as adsorption phenomena are dependent on extent of surface. We should therefore expect to get more marked changes in muscles having surfaces relatively large as compared with the total mass of the muscle; (2) previous activity of the muscle may also vitiate the results, as in the active muscle certain metabolic products are formed (doubtless by enzymic activity), which may increase the osmotic pressure of the muscle-plasma; (3) a third variation is sure to creep in, owing to seasonal differences in the constitution of the frog's blood and tissues. This seasonal variation is shown in the following data of absorption from solutions isosmotic with $\frac{1}{8}$m NaCl:

TABLE III

Season	Substance	Concentration	Absorption			
			1 h.	3 h.	6 h.	24 h.
Summer (Sept.)	$CaCl_2$	$\frac{1}{10}$ m	− 5	−10	−22	−19
Winter (Mar.)..	$CaCl_2$	$\frac{1}{10}$ m	+12	+18	+20	−10
Summer	NaCl	$\frac{1}{8}$ m	+ 0	+ 1	+ 2	+ 7
Winter.....	NaCl	$\frac{1}{8}$ m	+ 4	+ 4	+ 5	+ 6
Summer	KCl	$\frac{1}{8}$ m	+ 4	+12	+23	+39
Winter.....	KCl	$\frac{1}{8}$ m	+ 4	+ 7	+16	+55

In considering the results obtained in the absorption from cane-sugar solutions, we must take heed, therefore, of the concentration of the solution as well as its time of action. The chief question at issue, however, is whether we are dealing with a process explicable by the laws of osmotic pressure, or whether this process is due to some other physical or chemical change. From the above table of absorption from cane-sugar solutions, it will be noted that a muscle immersed in a hypertonic solution loses 53 per cent. of its weight in twenty-four hours, while in an isosmotic solution it gains 10 per cent., and in a hypotonic solution it gains 87 per cent. This cycle of absorption is exactly that which we should expect providing osmotic pressure effects were prominent. If a solution of salts be separated from a sugar solution by a membrane slowly permeable to the salts, quickly permeable to the water, and impermeable to the sugar, we should observe that water will pass from the salt solution into the sugar solution, providing the concentration of the sugar solution be greater than that of the salt solution. If the former be equal to or less than the latter, water will pass, to a less or great degree, from the sugar solution into the salt solution. This process is observed in phenomena of absorption from cane-sugar solutions.

As will be shown later, the sarcolemma of the muscle is slowly permeable to the ions of the salts within the muscle. Along with the passage of water from or into the muscle we will have, therefore, the passage of ions from the muscle into the sugar

solution. The ultimate result will be due, therefore, to a combination of osmotic pressure effects with those of diffusion.

c. Absorption from Urea Solutions.—The following table shows the absorption under influence of various concentrations of urea:

TABLE IV

Concentration	Isosmotic	Absorption			
	NaCl Sol.	1 h.	3 h.	6 h.	24 h.
2.0659 m.....	$\frac{5}{4}$ m	− 5	− 5	− 4	+ 4
1.6745 m.....	m	− 5	− 4	− 4	+ 2
.8667 m.....	$\frac{1}{2}$ m	− 3	− 2	+ 1	+15
.4465 m.....	$\frac{1}{4}$ m	+ 5	+14	+23	+42
.2286 m.....	$\frac{1}{8}$ m	+11	+29	+45	+72
.1168 m.....	$\frac{1}{16}$ m	+27	+46	+65	+66
.05906 m.....	$\frac{1}{32}$ m	+29	+42	+54	+53

It will be seen above that a muscle, immersed in a solution of urea isosmotic with $\frac{1}{8}$ m NaCl, gains in one hour 11 per cent., while in twenty-four hours the gain is 72 per cent. What do these facts denote? If the sarcolemma be impermeable to urea, as it was to cane-sugar, then, of course, we should have the phenomena controlled by laws of osmotic pressure. If it be permeable to urea, as well as to water, we should have, according to laws of diffusion, urea passing from point of higher to that of lower concentration, or, in other words, into the muscle. An effort would be made to adjust the equilibrium on both sides of this permeable membrane. Along with this partial adjustment of urea, must go the adjustment of the concentration of various ions on each side of the sarcolemma. A current of diffusion is thus set up, which passes in both directions through the sarcolemma. The result of all this would, therefore, be an increase in weight on the part of the muscle.

It is clear that osmotic or ionic effects cannot explain the large increase in weight under influence of urea solution. The progress of absorption from urea is, markedly, similar to that noted when water is used, although the absolute absorption in the latter case is more marked at each interval except the final one. As the results seem to show, urea easily penetrates the sarcolemma. Osmotic pressure effects cannot, therefore, be considered of the first importance. Just why such a marked increase in absorption occurs during the later intervals does not seem clear to the writer at present. It may be that certain decided changes, such as the breaking down of urea into NH_4 compounds or the formation of amido compounds, may result in an increased osmotic pressure and a resulting increase in absorption. The action of these nonelectrolytes upon phenomena of absorption is markedly different from that observed in other phenomena. Loeb, Lingle, Moore, Kahlenberg, True, and others have observed that, in general, nonelectrolytes have little or no effect on the phenomena under observation. In these experiments it is quite evident that the effect of the non-electrolytes is very marked.

VI. EFFECTS OF ELECTROLYTES

a. Halogen Salts.—In selecting the salts to be used in this investigation, attention was paid to the results previously obtained by Loeb. He had shown that the effect of the halogens was, practically, the same for any given metal, although the absorption increased slightly in the order: chlorides, bromides, iodides, and fluorides. It seemed unnecessary, therefore, to use in these experiments solutions of all the haloids. As the type of halogen salt, sodium chloride, was selected, and the solutions of other salts were made equimolar with the NaCl solutions. The concentrations of these solutions were, as previously stated, graded from $\frac{5}{4}$ m to $\frac{1}{32}$ m, in order to permit of study of variations due to differences of osmotic pressure of same salt.

At the outset of the work on the influence of electrolytes, the importance of the third factor, mentioned above as influencing the process of absorption, viz., the nature of the substance in solution, was observed. Van Bemmelen, it will be remembered, found that the absorption by SiO_2 depended, to a great extent, on the solution to be absorbed. In our work the nature of the cation, as well as of the anion of the salt used, influenced the absorption.

These facts are seen from the following table:

TABLE V

Salt	Concentration	Absorption			
		1 h.	3 h.	6 h.	24 h.
NaCl.....	$\frac{1}{8}$ m	+0	+ 1	+ 2	+ 7
KCl.....	$\frac{1}{8}$ m	+4	+12	+23	+39
NH_4Cl.....	$\frac{1}{8}$ m	+1	+ 3	+10	+29
$CaCl_2$.....	$\frac{1}{10}$ m	−4	−21	−24	−18
$MgCl_2$	$\frac{1}{10}$ m	+3	+ 7	+ 9	+18
Na_2SO_4....	$\frac{1}{10}$ m	+2	+ 6	+ 8	+ 6
K_2SO_4.....	$\frac{1}{10}$ m	+1	+ 3	+ 6	+ 7

It is noted from above that in a solution of NaCl the muscle gains only 7 per cent., in isosmotic KCl it gains 39 per cent., while in $CaCl_2$ solution it loses 18 per cent. The influence of the cation is seen when one compares the absorption under Na_2SO_4 and K_2SO_4 with that under NaCl and KCl. While the direct absorption under influence of the SO_4 ion is not, in itself, so marked, there must be some strong anion effect to offset the marked cation effect noted under action of KCl.

From the data of above table we see that the halogens of the alkali and of the alkaline-earth group are divided into two classes, according to their power of hindering or facilitating absorption of liquid by animal tissues. Ca ions stand out, prominently, as the one inhibiting absorption, while Na shows a slight effect only, and K, NH_4, Mg, SO_4, all have a favoring effect.

The following table gives a complete list of the various halogens used, together with their concentration and time effects. The figures given under each concentration are the average figures of six experiments each:

TABLE VI

Salt	Concentration	Absorption 1 h.	3 h.	6 h.	24 h.	Salt	Concentration	Absorption 1 h.	3 h.	6 h.	24 h.
NaCl.....	$\frac{5}{4}$ m	−23	−20	−10	+11	$CaCl_2$.....	$\frac{5}{4}$ m	−23	−22	−18	−18
NaCl.....	m	−17	−16	− 7	+ 9	$CaCl_2$.....	m	−21	−19	−15	− 5
NaCl.....	$\frac{1}{2}$ m	−13	−12	− 5	+ 6	$CaCl_2$.....	$\frac{1}{2}$ m	−18	−14	− 8	+ 2
NaCl.....	$\frac{1}{4}$ m	− 7	− 7	− 7	+ 3	$CaCl_2$.....	$\frac{1}{250}$ m	−16	−14	−12	− 1
NaCl.....	$\frac{1}{8}$ m	+ 0	+ 1	+ 2	+ 7	$CaCl_2$.....	$\frac{2}{8}$ m	−21	−18	−13	− 2
NaCl.....	$\frac{1}{16}$ m	+13	+22	+26	+41	$CaCl_2$.....	$\frac{1}{4}$ m	−18	−15	− 9	− 4
NaCl.....	$\frac{1}{32}$ m	+25	+42	+53	+75	$CaCl_2$.....	$\frac{1}{8}$ m	−15	−19	−21	−11
NaF	$\frac{1}{4}$ m	− 9	−13	− 7	+13	$CaCl_2$.....	$\frac{1}{8}$ m	−13	−17	−25	−22
NaF	$\frac{1}{8}$ m	+ 1	+ 4	+ 7	+22	$CaCl_2$.....	$\frac{1}{10}$ m	−12	−20	−26	−20
KCl.....	$\frac{5}{4}$ m	−18	−10	− 2	+21	$CaCl_2$.....	$\frac{1}{16}$ m	− 7	−26	−24	−22
KCl.....	m	−21	−15	− 6	+17	$CaCl_2$(Sept)	$\frac{1}{20}$ m	− 4	−21	−24	−18
KCl.....	$\frac{1}{2}$ m	−15	−11	− 3	+17	$CaCl_2$(Mar.)	$\frac{5}{20}$ m	+12	+18	+20	−10
KCl.....	$\frac{1}{4}$ m	− 5	− 5	+ 2	+19	$CaCl_2$.....	$\frac{8}{100}$ m	− 1	−17	−23	−14
KCl.....	$\frac{1}{8}$ m	+ 4	+12	+23	+39	$CaCl_2$.....	$\frac{1}{40}$ m	− 4	−18	−21	−15
KCl.....	$\frac{1}{16}$ m	+20	+40	+54	+56	$CaCl_2$.....	$\frac{1}{100}$ m	− 7	−22	−26	−17
KCl.....	$\frac{1}{32}$ m	+25	+50	+63	+70	$MgCl_2$	$\frac{5}{4}$ m	−18	−18	−11	+24
NH_4Cl.....	$\frac{5}{4}$ m	−12	− 5	+10	+20	$MgCl_2$	m	−15	−12	− 2	+30
NH_4Cl.....	m	−12	− 7	+ 4	+21	$MgCl_2$	$\frac{1}{2}$ m	−10	− 8	− 4	+30
NH_4Cl.....	$\frac{1}{2}$ m	−11	−12	− 6	+10	$MgCl_2$	$\frac{1}{4}$ m	− 5	− 1	+ 5	+32
NH_4Cl.....	$\frac{1}{4}$ m	− 6	− 8	− 6	+20	$MgCl_2$	$\frac{1}{8}$ m	− 3	− 3	− 1	+ 3
NH_4Cl.....	$\frac{1}{8}$ m	+ 1	+ 3	+10	+29	$MgCl_2$(Mar.)	$\frac{1}{20}$ m	+10	+21	+27	+36
NH_4Cl.....	$\frac{1}{16}$ m	+14	+30	+46	+67	$BaCl_2$(Mar.)	$\frac{1}{20}$ m	+12	+22	+30	+33
NH_4Cl.....	$\frac{1}{32}$ m	+27	+49	+66	+54						

B. NITRATES

Concerning the action of the nitrates, it is to be said that the NO_3 radical does not seem to differ, in its action, from the Cl ion, inasmuch as the absorption by a muscle immersed in a nitrate solution is of the same order of magnitude as the absorption by a muscle in the corresponding chloride solution. The conclusion is evident, therefore, that in the experiments with the salts of monobasic acids, the cation is the influencing factor, while the anion acts indifferently. Isosmotic solutions of the nitrates were used in all cases as the general effect of hyper-, iso-, and hypo-tonic solutions were identical with those noted under effect of chlorides.

TABLE VII

Salt	Concentration	Absorption 1 h.	3 h.	6 h.	24 h.
$NaNO_3$	.12568 m	+ 0	+ 3	+ 5	+ 7
KNO_3	.1258 m	+ 5	+12	+21	+50
NH_4NO_3...	$\frac{1}{8}$ m	+ 2	+ 5	+14	+34
$Ca(NO_3)_2$ ..	$\frac{1}{10}$ m	−10	−18	−24	−19

c. Sulphates.—In the experiments first performed, the concentrations of the sulphate solutions were made equimolar with the chloride solutions. Here the same general effects were noted as in the case of the monobasic salts, viz., marked loss of fluid when muscle was placed in hypertonic solutions, while in hypotonic solutions of the same salt a marked absorption was observed.

The effect of the cation, in combination with the anion SO_4, was shown by the earlier experiments to be very slight.

TABLE VIII

Salt	Concentration	Absorption			
		1 h.	3 h.	6 h.	24 h.
Na_2SO_4	$\frac{1}{8}$ m	−4	−6	−7	−10
K_2SO_4	$\frac{1}{8}$ m	−1	−2	−2	− 7
$(NH_4)_2SO_4$	$\frac{1}{8}$ m	−3	−4	−4	−10
Li_2SO_4	$\frac{1}{8}$ m	−4	−6	−7	−10

Inasmuch as these effects of equimolar solutions were so very nearly identical, the further experiments on effects of isosmotic solutions of the various sulphates were assumed to be unnecessary. The effect of isosmotic sulphate of sodium was, therefore, taken as a general effect of such solutions of sulphates. We notice from the above table, as well as the one given below, that the action of the sulphates seems to be dependent on the effect of the anion, while the effect of the chlorides and nitrates depends on the action of the cation.

Below is given a complete table of sulphates used together with their concentration and time effects:

TABLE IX

Salt	Concentration	Absorption				Salt	Concentration	Absorption			
		1 h.	3 h.	6 h.	24 h.			1 h.	3 h.	6 h.	24 h.
Na_2SO_4	$\frac{1}{256}$ m	−16	−19	−15	− 4	K_2SO_4	$\frac{1}{4}$ m	− 8	−11	−13	−12
Na_2SO_4	$\frac{1}{2}$ m	−16	−21	−18	−11	K_2SO_4	$\frac{1}{8}$ m	− 1	− 2	− 2	− 7
Na_2SO_4	$\frac{2}{20}$ m	−16	−23	−20	− 6	K_2SO_4	$\frac{1}{16}$ m	+ 8	+16	+24	+28
Na_2SO_4	$\frac{1}{4}$ m	−10	−12	−19	−17	K_2SO_4	$\frac{1}{32}$ m	+22	+41	+65	+67
Na_2SO_4	$\frac{1}{8}$ m	− 8	−12	−18	− 9	$(NH_4)_2SO_4$ ...	$\frac{1}{2}$ m	....	....	....	−12
Na_2SO_4	$\frac{1}{8}$ m	− 4	− 6	− 7	−10	$(NH_4)_2SO_4$ ...	$\frac{1}{4}$ m	....	....	....	−11
Na_2SO_4	$\frac{1}{10}$ m	+ 2	+ 6	+ 8	+ 4	$(NH_4)_2SO_4$ ...	$\frac{1}{8}$ m	− 3	− 4	− 4	−10
Na_2SO_4	$\frac{1}{16}$ m	+ 1	+ 8	+10	+13	$(NH_4)_2SO_4$ ...	$\frac{1}{10}$ m	+ 3	+ 7	+ 7	+ 3
Na_2SO_4	$\frac{1}{20}$ m	+10	+21	+28	+33	$(NH_4)_2SO_4$ ...	$\frac{1}{16}$ m	....	....	....	+20
Na_2SO_4	$\frac{1}{32}$ m	+12	+26	+35	+41	$(NH_4)_2SO_4$ ...	$\frac{1}{32}$ m	....	....	....	+44
Na_2SO_4	$\frac{1}{40}$ m	+25	+45	+55	+66	Li_2SO_4	$\frac{1}{8}$ m	− 4	− 6	− 7	−10
Na_2SO_4	$\frac{1}{100}$ m	+30	+50	+57	+48	$MgSO_4$	$\frac{1}{10}$ m	+10	+19	+26	+35
K_2SO_4	$\frac{1}{2}$ m	−13	−23	−26	−17						

d. Oxalates and Carbonates.—The absorption noted, when the influence of oxalate and carbonate solutions is studied, is as follows:

TABLE X

Salt	Concentration	Absorption			
		1 h.	3 h.	6 h.	24 h.
$Na_2C_2O_4$.....	$\frac{1}{4}$ m	−10	−12	− 6	+20
$Na_2C_2O_4$.....	$\frac{1}{8}$ m	− 4	− 6	− 7	+ 0
$Na_2C_2O_4$.....	$\frac{1}{16}$ m	+ 8	+12	+21	+30
$Na_2C_2O_4$.....	$\frac{1}{20}$ m	+12	+23	+31	+42
Na_2CO_3.....	$\frac{1}{4}$ m	0	+10	+21	+45
Na_2CO_3.....	$\frac{1}{8}$ m	+ 2	+15	+27	+53
Na_2CO_3.....	$\frac{1}{16}$ m	+12	+20	+28	+39
Na_2CO_3.....	$\frac{1}{20}$ m	+16	+30	+33	+45

From the above tables we see that the effect of the anions SO_4, C_2O_4, CO_3, is much different from that of the Cl anion. In equimolar solutions there seems to be a close analogy between the effects of the dibasic group and those of calcium salts of monobasic acids. These effects were to be expected if the idea of Wallace and Cushny, concerning the precipitation of calcium by saline cathartics, be correct. If, however, we compare isosmotic solutions, the apparent retarding effect of the anion is replaced by a favoring effect. Thus it will be observed that a muscle gains, in $\frac{1}{10}$m Na_2SO_4, 4 per cent in 24 hours, while in $\frac{1}{10}$m$CaCl_2$ it loses 20 per cent.

An apparent variation from the general effect of dibasic anions is noted in the case of Na_2CO_3 solutions. Here we notice that a positive absorption begins, at once, and increases much more rapidly than even that due to $\frac{1}{8}$m KCl solution. It will be remembered that Loeb found that the addition of a small number of OH ions greatly facilitated the absorption. In Na_2CO_3 solution we have quite a marked hydrolysis, giving us free OH ions, upon which depends the alkaline reaction of the solution. We are, therefore, justified in assuming that the marked positive effect, noted in case of Na_2CO_3, is due to the free OH ions present in the solution.

e. Citrates.—The results following the use of citrate solutions were, practically, the same as those observed with dibasic salts. Here, again, we notice that isosmotic solution ($\frac{1}{12}$m) of sodium citrate has a slightly favoring effect, instead of an inhibiting one. Although the absorption under the influence of dibasic and tribasic salts is not at all great, yet the variation, noted in the effects of Na and K salts of monobasic acids, is lost, when we observe the results of absorption under Na and K salts of polybasic acids. The anion must, therefore, exert some influence on the absorption, either by neutralizing the effect of the cation as in the case of K_2SO_4 or, perhaps, by changing the state of the muscle plasma by the precipitation of calcium.

TABLE XI

Salt	Concentration	Absorption			
		1 h.	3 h.	6 h.	24 h.
$Na_3C_6H_5O_7$ ...	$\frac{1}{8}$ m	− 6	− 9	−10	− 9
$Na_3C_6H_5O_7$ ...	$\frac{1}{12}$ m	+ 4	+ 4	+ 3	+ 3
$Na_3C_6H_5O_7$ ...	$\frac{1}{80}$ m	+11	+20	+28	+39

VII. EFFECTS OF TEMPERATURE ON ABSORPTION PHENOMENA

Van Bemmelen found, in his work on inorganic colloids, that the power of absorption decreased with the temperature. This, he explained, was due to a change in the structure of the colloid, which change resulted in the transformation of the colloid into a much denser physical state. He says that every change in structure of a colloid causes a change in the absorption power of a colloid, if this structure be non-reversible when brought into contact with a solution. All such modifications cause (1) a thickening or drawing together of the walls of the colloid, (2) a narrowing of spaces in the substance of the colloid, (3) a change of capillary force, and (4) a consequent change of the absorption coefficient of the colloid.

As we are dealing with a non-reversible gel, the protoplasm, we should expect to find that any modification, brought about by temperature, in the muscle, would show the decrease of absorptive power spoken of above.

Our first experiments bearing on this point were made with temperatures easily found in the laboratory. The muscles were placed in the solutions and allowed to remain at the same temperature for 24 hours, at end of which time they were weighed and their absorption calculated.

The results of these first experiments showed that, in the range of temperatures found in the laboratory, absorption effects were practically the same for the same salt and same concentration of this salt. We, therefore, arranged a series of experiments in which the muscle was put into rigor by being heated to 50–52°C in a solution of $\frac{1}{8}$m NaCl for 10 minutes. After being thus treated the muscle is dried, weighed, and placed in certain solutions for 24 hours. Control experiments were made with muscle not in rigor. The striking effects may be observed from the following table.

From above data it is quite evident that some change has occurred in the muscle which lessens the power of absorption. The result is most striking in cases of $\frac{1}{8}$m KCl and $\frac{1}{8}$m $CaCl_2$. We see here that a muscle in rigor absorbs only a very slight amount of fluid if placed in a KCl solution while in a $CaCl_2$ solution a much greater absorption or, rather, a smaller negative absorption is observed. The conclusions are quite evident that some structural change has occurred in the muscle which modifies the absorptive power of the muscle. Comparing this result with those of van Bemmelen we are led to believe that the structure of the muscular protoplasm is much like that of the gel of SiO_2, viz., a web-like structure of colloid, holding, in its micellary and

TABLE XII

Salt	Concentration	State of Muscle	Absorption in 24 Hours
KCl.....	$\frac{1}{8}$ m	Rigor	+ 3
KCl.....	$\frac{1}{8}$ m	Normal	+45
NaCl.....	$\frac{1}{8}$ m	Rigor	+ 8
NaCl.....	$\frac{1}{8}$ m	Normal	+ 8
NaCl.....	$\frac{1}{4}$ m	Rigor	+11
NaCl.....	$\frac{1}{4}$ m	Normal	+27
NaCl.....	m	Rigor	+ 9
NaCl.....	m	Normal	+20
$CaCl_2$.....	$\frac{1}{8}$ m	Rigor	− 3
$CaCl_2$.....	$\frac{1}{8}$ m	Normal	−22

interstitial spaces, fluid by capillary attraction, adsorption, and absorption. The recent experiments of Greeley confirm this idea of change of structure under influence of temperature and consequent change in absorptive power of muscle.

VIII. THEORETICAL CONSIDERATIONS

I. PERMEABILITY OF SARCOLEMMA

In discussing the theoretical conclusions to be drawn from the experiments detailed in this paper, the question to be first settled is whether the protoplasm of the muscle is permeable and if so in which direction or directions and to what substances.

It will be remembered that in former papers Lingle, Loeb, and Miss Moore called attention to the fact that the rhythmical contractions of strips of turtle's ventricle, of skeletal muscle, and of the lymph hearts of frogs, were under the influence of certain ions. They further showed that electrolytes caused an effect while non-electrolytes caused none. Loeb's explanation of the phenomenon noted in skeletal muscle was that a certain physiological balance existed between the inorganic cations of the muscle plasma. If this balance be disturbed certain phenomena, among them rhythmical contractions of the muscle, are observed. He shows, as do also the other authors noted above, that the contractions are due to the excess of Na ions beyond that physiological relation existing between Na and Ca. If a muscle be immersed in a sodium chloride solution Na ions, says Loeb, will penetrate the muscle and will gradually replace the Ca, K, and other cations present. In about an hour rhythmical contractions will begin and will last for some time. If, instead of an NaCl solution, a Na_2SO_4 solution be used, these contractions appear at once, because, as he says, the physiological relation has been disturbed both by entrance of Na ions and by the precipitation of the Ca ions by SO_4 radical. This latter result is more striking if an alkaline oxalate or citrate solution be used instead of a sulphate solution. It was further shown by these authors that Ca, K, Mg, Ba, etc., had exactly the opposite effect upon rhythmical contractility, viz., an inhibiting action. It seems quite evident, from these

results, that we must admit the permeability of the muscle membrane to the anion as well as to the cation of the salts investigated. However, it must be said that the effect of the anion seems to have no greater significance than that those anions precipitating Ca act much more readily than the others by disturbing the physiological balance more quickly than do the anions Cl, Br, I, NO_3, etc.

Concerning the permeability of the sarcolemma to non-electrolytes such as sugar, urea, water, etc., we have little literature to draw from. The above mentioned authors show that rhythmical contractions do not arise in solutions of non-electrolytes. However, from the absorption phenomena noted in case of cane sugar and urea we notice that the sarcolemma is permeable to urea and not to cane-sugar solutions.

Having shown the permeability inward of the sarcolemma for the various substances investigated, our next question seems to be, Is the sarcolemma permeable outward to these same substances? It is a well-known fact that the red blood-corpuscles of most animals contain more potassium than sodium while the serum of the same animal contains these elements in the reverse relation. This same thing is noticed in case of composition of muscle plasma. These facts seem to show that the corpuscles and muscles are impermeable to the Na and K ions, else we should have an equilibrium established between these ions on both sides of the membranes in question. The work, previously mentioned, on red blood-corpuscles seems to show that the corpuscular membrane is impermeable to K, Na, Ca, sugar, etc., while it is permeable for NH_4Cl, urea, etc. Our work on muscle shows that the sarcolemma is permeable for Na, K, Ca, NH_4, urea, etc., while it is impermeable to sugar. How account for these variations? As we have seen above, differences in osmotic pressure were given as the cause of the phenomena noted in red blood-corpuscles, while, in our work, these differences do not explain the effects of the various substances used. The answer to these questions might be that in case of blood corpuscles we are dealing with a membrane which is permeable in neither direction to certain ions, while in muscle we have to do with a membrane permeable in both directions although the rate of diffusion is different for each ion and is different in the two directions. That this latter statement is correct may be readily seen from the following: The permeability inward of the sarcolemma is evident from the experiments on rhythmical contractions of muscle. If a muscle be placed in a solution of $CaCl_2$, as we have seen, Ca and Cl ions will enter the muscle and will inhibit or restore rythmical contractions of a muscle according to the state of a muscle previous to immersion in the solution. It can be definitely shown that K and, possibly, Na ions have penetrated outward from the muscle by a simple qualitative chemical test of the solution in which the muscle has been immersed. By addition to this solution of hydrochlorplatinic acid, evaporation of the mixture nearly to dryness, and then the addition of alcohol, we obtain the characteristic reddish-yellow octohedra of K_2PtCl_6. It is to be remembered here that the detection of Na in presence of K is not possible by this test as the Na_2PtCl_6 is soluble in the alcohol used. We can, therefore, state definitely that K ions have penetrated outward and that in all probability the Na ions have also done the same. Do Ca ions also

penetrate outward? If a muscle be placed in a solution of Na_2SO_4 rhythmical contractions are, as mentioned above, observed at once. If the muscle be allowed to remain in these solutions 12 hours a slight opalescence is observed which becomes more marked at the end of 24 hours and may even pass into a distinct precipitate. On examination of this precipitate by ordinary qualitative methods, Ca was, unmistakably, shown by its characteristic oxalate. There can be no doubt, therefore, of the permeability outward of the sarcolemma in case of Ca ions. This latter reaction must not be confused with another which is observed in practically all of the salt solutions used, although it was more marked in case of the solutions whose anion precipitated Ca. The reaction in question is as follows: If a muscle be placed in certain salt solutions, preferably the sulphate or oxalate of alkali metals, there will be observed in 18 hours a decided opalescence. This turbidity is not all due to the precipitation of Ca salts as is seen from the experiment. The opalescent solution is heated to 65–75° C for a few minutes, when a distinct coagulum appears which resembles that of white of egg. The solution has a strong proteid odor and shows a distinct froth on its surface. Tested with $CuSO_4$ and KOH a distinct violet coloration is noted. This seemed to point to the presence of proteid matter in the solution. In order to confirm this assumption it seemed necessary only to test for carbon and nitrogen. By the ordinary organic methods of examination both these elements were detected, showing conclusively the presence of proteid. Here we have, evidently, to do with the solvent action of the salt solution upon the proteid matter of the muscle. We are, however, not in a position to give the origin of this proteid. Two possibilities present themselves for consideration. In the first place we may have a simple solvent action of the salt solution upon the surface of the muscle. Secondly, we might conceive of some combination of the proteid, within the sarcolemma, with the salt, which combination is diffusible. The first possibility seems much more plausible but cannot be absolutely proven by these experiments.

The sarcolemma of the muscle is, therefore, permeable to the ions under investigation. However, it is readily seen from the previous points that the rate of movement of the ions is different in each direction. Whereas, for instance, the inward penetrability of the Na ion may be definitely shown within a few minutes, the outward penetrability may not be detected for several hours. Moreover, we know that the rate of diffusion of Ca ion is much slower than that of the Na and approximately the same as the K ion because, according to Graham's law of diffusion of gases, we should expect the rate of diffusion to be inversely proportional to the square root of the density of a solution. Reid has shown that the rate of osmosis through the skin of a frog is different in each direction. We are, hence, justified in our assumption here, that the sarcolemma of the muscle is permeable in both directions to certain ions and that the rate of diffusion is different in each direction being much faster inward than outward. In this passage of ions into and out from a muscle we have many more ions entering the muscle than leaving during the same interval of time owing to the factors (1) of rate of migration of ion and (2) direction of migration. Just how far these assump-

tions affect the permeability of the muscle for non-ionized substances, such as cane sugar and urea, we are not in a position to state, as no direct experiments were made bearing on the point of outward penetrability of urea. Cane sugar, it will be remembered, does not seem to penetrate the sarcolemma at all and therefore obeys perfectly the laws of osmotic pressure.

II. OSMOTIC PRESSURE EFFECTS

a. At the beginning of this section it may be asserted that differences in osmotic pressure, on both sides of the sarcolemma, do not account for the results of absorption of fluid by muscle. Hamburger's work on the red-blood corpuscle apparently proved that such was the case in phenomena noted in his experiments. He, however, neglected the consideration of partial osmotic pressure as well as the question of permeability of corpuscular membrane to certain ions, in which latter case osmotic pressure could play no rôle whatever. From our own experiments on blood-corpuscles we find, by use of Hedin's hæmatokrit method, that the corpuscles do, apparently, obey osmotic laws if solutions of K, Na, Ca, etc., salts be used. Yet we cannot assert from these experiments that these laws are the only ones governing the phenomena noted, inasmuch as our work on the muscle shows us, conclusively, that osmotic effects are accountable for slight changes only.

b. Generally speaking, a muscle immersed for a short interval in a hypertonic solution of an electrolyte or non-electrolyte will lose in weight; if placed in an isotonic solution it may or may not gain, while in a hypotonic solution of the same substance it regularly increases in weight. Miss Cooke has found, it will be recalled, that in a solution of $\frac{1}{8}$ m NaCl (5.1087 atmospheres pressure) a muscle neither gains nor loses in weight in eighteen hours. The assumption was very natural that this NaCl solution was isosmotic with the muscle plasma, as Loeb's previous work showed that the effects of acids and alkalies were proportional to the number of free H and OH ions present in the solutions. This assumption is, however, open to several serious objections. In the first place it has been shown by Pfeffer, Linebarger, Picton and Linder, Sabanejew, Tamman, and others, that colloids have, in solution, slight osmotic pressure values. In the second place, as we have shown above, Na, K, Ca, SO_4, Cl ions penetrate the sarcolemma in both directions though at a varying rate in each direction. We must, therefore, conclude that the laws of osmotic pressure do not explain the phenomena noted in these absorption experiments. The markedly different effects of isosmotic solutions of various chlorides show us that the ionic effects far exceed the osmotic effect noted in these experiments.

c. If the question of interchange of ions or molecules through animal membranes be considered, we find that the relative semi-permeability to ions must determine whether or not osmotic pressure laws are valid in such experiments. Our experiments show clearly that we are dealing in this phenomenon of absorption of fluid by muscle with a combination of ionic effects on the one hand with those of osmotic pressure and hydro-diffusion on the other. The laws of diffusion state that substances, obeying

these laws, pass from the point of higher to that of lower concentration of these substances and, of course, show a greater velocity the higher the temperature. A free interchange of ions between the solution outside and that inside the sarcolemma is naturally prevented by the relative permeability of sarcolemma as well as the varying velocity of the ions in the two directions. As the rate of diffusion is dependent on the density of the solution, the amount of fluid passing into a muscle from a hypertonic solution would be much less than that entering from a hypotonic solution. Such is the case as observed in our experiments, yet it is more than probable that osmotic effects play a much greater rôle in these stronger and weaker solutions than in isosmotic solutions where the ionic effects are more marked.

Just how far the sarcolemma influences the process of diffusion cannot be stated, inasmuch as our knowledge of the structure of this membrane is meager. The views of Klein and van Beneden concerning the reticular structure of protoplasm are opposed by those of Bütschli, who advances the theory of "foam-structure" of protoplasm. Recent work by Hardy upon organic colloids and van Bemmelen upon inorganic colloids show that the structures with which they were dealing in their experiments were web-like structures in the interstitial spaces of which fluid is held by capillary attraction, adsorption, and absorption.

Brücke has advanced a theory of "pore diffusion" to explain the effect of an animal membrane upon diffusion. He assumes capillary spaces in the membrane, which spaces hold a layer of liquid by capillary attraction. If the space be very small, then, of course, the membrane becomes relatively semi-permeable. Fick, in addition to this idea of pore diffusion, assumes that this process occurs by a diffusion through the molecular aggregates making up the membrane. This latter process is, of course, dependent on laws of adsorption.

We are, therefore, not in a position to say exactly what influence the sarcolemma exerts upon the absorption noted in our experiments, but we can state that a much more complicated process is involved here than would be the case if the sarcolemma were absolutely impermeable to the substances used.

d. The element of time plays an important rôle in the phenomena observed in our experiments. The effect during the first intervals is much more marked, relatively speaking, than during the later ones and is also, in hypertonic solutions, of a different phase. What do these variations denote? We might assume that the physiological condition of the sarcolemma and of the muscle as a whole has been markedly affected by the solution used. This assumption is borne out by Reid, who showed that the passage of fluid through the skin of the frog is intimately connected with the physiological condition of the tissue. He further showed that agents which tend to depress the vital activity diminish the osmosis in the normal direction, while those agents stimulating activity give rise to an increase in osmosis. It has also been shown by Loeb, Lingle, Moore, and Kahlenberg and True that certain salts act as definite protoplasmic poisons. Loeb pointed out that pure solutions of NaCl, KCl, $CaCl_2$ are poisonous as far as contractile power of muscle is concerned. Miss Moore, in her work on trout

and on the lymph-heart of frogs, showed that the solutions mentioned above have poisonous effects in these latter cases. We are, therefore, supported in the assumption that the time effects may be due to certain physiological changes in the muscular tissues resulting, possibly, in formation of various products of enzymic activity, which products would increase the osmotic pressure inside the muscle and therefore cause a later absorption of fluid.

III. IONIC EFFECTS

(a) If a muscle be immersed in a $\frac{1}{8}$m NaCl solution for twenty-four hours it gains but slightly in weight. In a solution of $\frac{1}{8}$m KCl (isosmotic with $\frac{1}{8}$m NaCl) the gain is 40 per cent., while in a solution of $\frac{1}{10}$m CaCl the loss is 20 per cent. From the previous sections of this paper the variations in the effects of cations Na, K, NH_4, Ca, Mg, Ba, and anions SO_4, C_2O_4, $C_6H_5O_7$, CO_3, Cl, and NO_3 will be evident. It was shown above that osmotic pressure differences could not account for the divergence noted when isosmotic solutions of various salts were used. Loeb, in his work on absorption by muscle, advanced the following theory to explain the phenomenon noted at the beginning of this section. "Salts or electrolytes in general do not exist as such in the living tissues. In the muscle the various metal ions exist in combination with the proteid, in which combination they may be easily substituted, one for the other. In this substitution certain physical properties of colloid, especially their power of absorption of water and their state of matter, are changed." According to this "ion-proteid" theory, we should expect that, no matter what the outside solution might be, the ions of this solution would enter the muscle and substitute themselves for the ions present in the muscle. It is a well known chemical fact that we have combinations of metal ions with the radicals of the higher fatty acids forming a distinct class of compounds known as soaps. Potassium soaps absorb large amounts of water, in fact they constitute the class of soft-soaps, which have an almost fluid consistency. Sodium soaps absorb only a slight amount of water, while the calcium soaps are very insoluble and absorb none. The effects of ions upon absorption by muscle are to form "ion-proteids," which effects show a "remarkable parallelism with the influence of the same ions upon absorption of water by soaps." The effects of Ba and Mg ions are quite different from those of Ca ions. Chemically speaking, we might expect these former ions to behave like Ca ions, yet, physiologically considered, effects do not always follow chemical characteristics. Our first idea of the cause of this variation was that, inasmuch as $MgCl_2$ is hydrolyzed in solution to MgOHCl and HCl, we might be dealing here with an action of the H ions of the HCl. This is not a very plausible conclusion, as the hydrolysis is so slight that such a marked result could hardly follow. This was further shown to be untenable when the action of $BaCl_2$ was investigated. This salt is not at all hydrolyzed in solution and can therefore have no free H ions in its solution. As the effects of $BaCl_2$ and $MgCl_2$ are almost identical, we must assume that we are dealing with the same cause in each case. We therefore revert to the ion-proteid theory and conclude that Ba- and Mg-proteid compounds show much the same absorptive power as do the K-proteid combinations. That Ba and Mg soaps show this

same property of absorbing water may be doubted, as the soaps of the alkali-earth metals are all insoluble and show no phenomena of solid solution.

(*b*) The effect of the anion bound to the cation shows a marked variation in cases of Cl and NO_3, on the one hand, and the SO_4 group on the other. From previous data we notice that the ions of Cl, Br, I, and NO_3 influence, to a very slight extent, the absorption of fluid by a muscle, while those anions which precipitate calcium show a marked positive effect. In fact, in solutions of salts of the polybasic acids, used in our experiments, the anion was the all-powerful factor, while the cation plays an indifferent rôle. In isosmotic solutions of the sulphates (the metal group being indifferent) the absorption is between 4 and 10 per cent., agreeing, practically, with the absorption under influence of NaCl.

We must, therefore, assume that in this ion-proteid combination the anion, as well as the cation, is attached to the proteid molecule, but at a different point of the molecule. It thus appears that the group of salts, known pharmacologically as saline cathartics, is not, in reality, an inhibitor of absorption, but is rather an accelerator of this process. As the calcium salts seem to have a direct inhibiting effect on absorption by muscle, it may be possible that these cathartics may, by their precipitating action on the calcium contained in the muscle, exert an accelerating effect. This conclusion is hardly plausible, inasmuch as we have the same amount of absorption under the influence of NaCl.

(*c*) The assumption, therefore, of a combination of both anion and cation with the proteid of the muscle seems to be the most valid one. We must, however, admit that in this combination the effects of the anion are in some cases more prominent, while in others the action of the cation is the important factor.

That cations and anions both combine with the proteid has been directly shown by Pauli, Tangl and Bugarsky, Spiro, Atkinson, Stewart, and others. Our assumption of "ion-proteids" is therefore made valid by direct experimentation.

IV. INDEPENDENT ABSORPTION OF SALT AND WATER

The question naturally arises in this discussion, whether the increase in weight of a muscle immersed in a solution is due entirely to absorption of water or partly to water and partly to dissolved substance. Hofmeister has definitely shown in his work on absorption of solutions by gelatine disks, that an independent absorption of salt and of water takes place. That is, the solution is not absorbed as such, but water is absorbed up to a certain limit, depending on the concentration, while salt is taken up in quantities approximately proportional to concentration. Our work was not planned to show the dynamics of the absorption to any further extent than to prove the absorption of water on the one hand, or, on the other, the absorption of the salt along with the water.

It will be recalled that the sarcolemma is permeable to certain ions which have been shown to actually enter the muscle and form compounds with the proteid (colloids) of the muscle. We should, therefore, expect to find by gravimetric methods that salts had actually added to the original weight of the muscle. If we estimate the

dry weight of one gastrocnemius by placing it, as soon as it is removed from the body, in a dessicator containing sulphuric acid, for twenty-four hours, while the dry weight of the other gastrocnemius of the frog is estimated after it had been in a solution of $\frac{1}{20}$m Na_2SO_4 for twenty-four hours, we should expect the dry weight of these two muscles to show the same slight variations noted in the original muscles, provided the increase in weight of the second muscle was due entirely to taking up of water. If an absorption of salt occurred in the second muscle, this absorption would of course be evident in an increase of the dry weight of the second over the first muscle. Such experiments were carried out with several muscles, the result being quite positive in all cases. From the following data the absorption of salt may be readily observed:

Wt. of orig. muscles in grams	Wt. after being in sol. 24 hrs.	Weight after drying 36 hrs.	Orig. difference in wt.	Final difference in wt.
2.313	3.42	.6347	.0189	.0315
2.2941		.6032		

The original difference in weight is markedly less, relatively speaking, than the final difference. Apparently, an absorption of .0126 grams of salt has taken place. It seems very plausible to conclude, from this composite experiment, that absorption of both water and salt has occurred but that a much larger absorption (98 per cent.) of water than of salt has taken place. If the solvent action of the solution upon the proteid, as well as the outward passage of Ca and other ions from the muscle, be remembered, it may be readily understood why the increase in dry weight is not more marked. It may be argued that the time of drying would necessarily influence the amount of water given off by the muscle when in the dessicator and that this slight increase was due to unequal time of evaporation. The experiment was continued for some time, daily observations being made, until the weights of the two groups of muscles remained constant for two consecutive days. We may in this case assume that the difference in weight, if any exists, is due to the taking up of salt by muscles immersed in the salt solution. The results follow:

Original weight	Wt. after being in sol. 24 hrs.	Dry wt. after 11 days	Orig. difference in wt.	Final difference in wt.
2.313	3.42	.4702	.0189	.0341
2.2941		.4361		

The difference has not disappeared on more complete drying. An interesting observation was brought out in these experiments. If we calculate, from figures given by Danilewsky, the amount of water in the above muscles, we find that the weight obtained by deducting the amount of water from the weight of the original muscle is larger than the weight obtained in our drying experiment. It is therefore clearly evident that Danilewsky's figures for per cent. of water in muscl are low or that in these experiments some abnormally watery muscles were used. In the following data these points may be noted, assuming, according to Danilewsky, 78.8 per cent. H_2O in muscle:

Original weight	Amount of H_2O 78.8 per cent.	Weight after deducting H_2O	Weight after drying
2.313	1.823	.4900	.4702
2.2941	1.808	.4861	.4361

IX. SUMMARY AND CONCLUSIONS

1. Muscles absorb fluid from hyper-, iso-, and hypo-tonic solutions of electrolytes and non-electrolytes.

2. The sarcolemma of the muscle is permeable to the ions Na, K, Ca, Mg, Ba, NH_4, Li, Cl, Br, I, NO_3, SO_4, C_2O_4, CO_3, $C_6H_5O_7$, in both directions, but at a much slower rate outward than inward.

3. Osmotic effects account only for absorption from water and cane sugar solutions.

4. Specific ionic effects play an important role in absorption from solutions of electrolytes. The "ion-proteid" theory seems to fully explain all cases noted.

5. The effect of the anion is marked in solutions of the salts known as saline cathartics. Instead of an inhibiting effect on absorption, these anions favor this process to a certain degree.

6. Absorption from isosmotic solutions depend on (1) the nature of the substance in solution, (2) time of action of the solution, (3) relative permeability of sarcolemma, (4) physical state of the muscle, and (5) temperature at which absorption takes place.

7. K, NH_4, Ba, and Mg cations seem to favor absorption, Na and Li are indifferent, while Ca cations show a marked inhibiting action on this process.

8. The anions Cl, Br, I, and NO_3 act indifferently toward the process of absorption, while anions of the SO_4 group show a positive effect.

In conclusion I wish to thank Professor Loeb for his many valuable suggestions both as regards methods of experimentation and interpretation of the results obtained.

ADDENDUM

SINCE the manuscript of the above article was sent to press, two articles by E. Overton on allied subjects, have appeared: "Beiträge zur allgemeinen Muskel- und Nervenphysiologie," *Archiv f. d. ges. Physiol.*, Vol. XCII (1902), pp. 115 ff.; *ibid.*, pp. 346 ff.

These articles, although bringing out certain facts mentioned in above paper, show that Overton is not familiar with the entire literature of the subject.

It is hard to explain the known facts concerning nutrition, as well as those previously brought out by Loeb on rhythmic contraction of muscle under influence of certain ions, without granting the permeability of the muscle plasma for the ions in question. This fact is absolutely contradicted by Overton.

The second paper, dealing with the influence of Na ions on muscle contractility, is, on the whole, a confirmation of Loeb's work published in 1899 and 1900.

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